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Immunoglobulin G Avidity Testing in Serum and Cerebrospinal Fluid for Analysis of Measles Virus Infection

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We studied a variety of patients with measles virus infection by using avidity testing for measles virus-specific immunoglobulin G (IgG) in serum and cerebrospinal fluid samples. For the avidity testing, an Enzygnost measles IgG enzyme-linked immunosorbent assay kit was used with an 8 M urea denaturing method. With this method, low-avidity IgG (acute primary infection, avidity of <30% within 15 days of the onset of rash) and high-avidity IgG (subacute sclerosing panencephalitis, avidity of >75%) could be clearly distinguished by using serum samples. One patient, who developed a typical course of measles despite a previous vaccination, showed a positive IgM response with an initial low titer of measles virus-specific IgG of low avidity, but a later sample revealed a high titer of IgG of intermediate (40%) avidity, suggesting previous immunological priming. Two patients with breakthrough infection (secondary vaccine failure), both having central nervous system involvement, showed a positive IgM response with initial high titers of serum IgG of high avidity. In addition, one of the patients had a detectable level of measles-specific IgG in cerebrospinal fluid. In this patient, the avidity of both serum and cerebrospinal fluid IgG decreased during the short follow-up period. This phenomenon has never before been reported. In subacute sclerosing panencephalitis patients, the avidity of cerebrospinal fluid IgG was consistently lower than that of serum IgG. The difference in avidity between cerebrospinal fluid and serum IgG may be used as a direct indicator of intrathecal production of IgG. In conclusion, the avidity testing is simple to perform, reliable, and highly informative in the analysis of measles virus infection.

Recent studies with measles virus as well as some other infectious agents have revealed the diagnostic usefulness of avidity testing for agent-specific immunoglobulin G (IgG) in serum (1, 2, 5, 7, 9, 12, 15, 27, 28, 30, 32). Avidity is a term which implies functional affinity of IgG class antibody, which is low during the first few weeks following primary infection and increases contemporaneously with the maturation of IgG. In particular, evaluating the avidity of the IgG antibody is expected to be superior to detecting IgM antibody in assessing disease characteristics of persistent infection, recurrent infection, or reactivated disease, since it has been shown that the IgM antibody is detectable in serum for a longer time than was previously appreciated and is detectable in recurrent infection as well (2, 5, 7, 9, 15, 28, 32). Here we report our results of avidity testing for measles virus-specific IgG in cerebrospinal fluid (CSF) as well as serum samples from a variety of patients with measles virus infection. These data clearly indicated that avidity testing provides information which is useful not only for diagnosis but also for further evaluation of central nervous system (CNS) complications associated with measles.

MATERIALS AND METHODS

Patients. Patients' characteristics are summarized in Tables 1 through 3. Patients 1 through 7 had not received a measles vaccination and ran a typical course of measles which was compatible with the Centers for Disease Control case definition of measles (3). Patient 8 had been under a combined immunosuppressive therapy against serious purpura nephritis (a combination of azathioprine [2 mg/kg] and prednisolone [1 mg/kg of body weight]) and developed fever 11 days after accidental contact with a measles patient in a hospital ward. The illness was complicated by bronchopneumonia and she eventually died from pulmonary insufficiency. Although neither skin rash nor Koplik spots were observed, a diagnosis of measles was confirmed by serology (a hemagglutinin inhibition titer rose from less than 1:4 to 1:256) and by a positive result of PCR (20) carried out with a peripheral blood mononuclear cell sample. Patient 9 had been vaccinated with the CAM strain at the age of 1 year 6 months but ran a typical course of measles with Koplik spots. Patient 10 had been vaccinated 14 days before the onset of rash (strain not specified). Although Koplik spots were not observed, the hemagglutinin inhibition titer rose from less than 1:4 to 1:64. Case 11 was recorded in 1979 as atypical measles, and further information could not be obtained. Classically, vaccine failure has been classified in two categories, that is, primary vaccine failure (PVF) (failure to elicit primary immunological response) and secondary vaccine failure (SVF) (a breakthrough infection). From the clinical point of view, in general, the clinical course which is indistinguishable from natural measles with Koplik spots represents PVF, and milder clinical manifestations such as short duration of fever, a skin rash in a limited region, and the absence of Koplik spots represent SVF (4). Also, some degrees of measles-like disease are known to occur following live attenuated measles vaccination. In this respect, on the basis of clinical presentation, case 9 was attributed to PVF, case 10 was attributed to vaccine measles, 11 was attributed to atypical measles, and cases 16 and 17 were attributed to SVF. Cases 12 through 15 were complicated by acute encephalitis, the signs and/or symptoms of which were manifested within 15 days of the onset of rash. Case 16 was a problematic case having a severe CNS complication despite a previous history of vaccination with the CAM strain at the age of 1 year 6 months and was therefore described in detail elsewhere (22). Case 17 was similar to case 16 in that there was a previous history of vaccination (strain not specified) at the age of 1 year 6 months and Koplik spots were absent. The patient recovered uneventfully without neurological sequelae. Cases 18 through 24 were cases of subacute sclerosing panencephalitis (SSPE). The diagnosis of SSPE was made according to the clinical manifestations, electroencephalographic findings, and high titers of anti-measles virus hemagglutinin inhibition and/or complement fixation tests in CSF (13).

All of the samples had been stored at either −20°C or −80°C for as long as 16 years. During our previous studies using PCR to detect measles virus genome (20) and quantitation of cytokines (21) some of the samples were defrosted up to three times. For this reason, we retested one sample (a serum sample from patient 21) after more cycles of freeze-thawing (a total of six times and a total of nine times, respectively).

Method. Measles virus-specific IgM was detected with an Enzygnost measles IgM enzyme-linked immunosorbent assay (ELISA) kit (Behringwerke, Marburg, Germany) (24) according to the supplier's instructions. For IgG, an Enzygnost

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TABLE 1. Avidity of serum IgG in patients with measles

Patient	Age	Sex[a]	Presence of Koplik spots	No. of days from onset of rash[b]	Result of IgM detection[c]	IgG	
						Titer (100)[d]	Avidity (%)
1	14 yr	F	+	1	Neg	ND	
				6	Pos	22	14
2	14 yr	M	+	4	Pos	ND	
				7	Pos	6	26
3	7 mo	M	+	1	Pos	ND	
				4	Pos	ND	
4	2 mo	F	+	0	Neg	ND	
				15	Pos	11	17
5	12 yr	M	+	1	Pos	3	29
6	8 yr	M	+	−1	Neg	ND	
				5	Pos	18	0
7	1 yr	M	+	−3	Neg	ND	
				1	Pos	3	0
8	5 yr	F	−	26[e]	Pos	67	17
				38	Pos	71	24
9[f]	10 yr	M	+	1	Pos	26	16
				8	Pos	527	40
10[g]	1 yr	M	−	3	Pos	ND	
				10	Pos	12	1
				16	Pos	61	2
11[h]	14 yr	F	−	1	Neg	187	46
				9	Neg	220	45

[a] F, female; M, male.
[b] The day of onset of rash was considered day 0.
[c] Neg, negative; Pos, positive.
[d] ND, not detected.
[e] Number of days from the onset of fever.
[f] Vaccinated at the age of 1 year 6 months.
[g] Vaccinated 14 days before the onset of rash.
[h] Atypical measles.

measles IgG ELISA kit (Behringwerke) (26) was used. After appropriate dilution, each sample was divided into two portions, and these were incubated in the wells for 1 h at 37°C. Following this, one sample was washed twice for 5 min each time with a wash solution which was contained in the kit. The other sample was subjected to washing (twice, each time for 5 min) with a wash solution which was supplemented with 8 M urea (2, 5, 15, 27, 30, 32) (Nacalai Tesque, Kyoto, Japan) followed by rinsing (twice, each time for 2 min) with the ordinary wash solution. After 1 h of incubation at 37°C with the peroxidase-conjugated anti-human IgG, the plate was washed with the ordinary wash solution. The substrate-chromogen mixture was added, the plate was incubated for 30 min at 25°C, and at the end of the incubation the stop solution was added. All procedures were done manually. The A_{450} was measured with a microplate reader (model Vmax; Molecular Devices, Sunnyvale, Calif.), and the titer of IgG was calculated. Avidity was calculated as the ratio of the absorbances with and without urea treatment (27, 30).

RESULTS

The results of detection of IgM and the calculation of IgG titers and avidity of IgG are presented in Tables 1 through 3. Freeze-thaw cycles did not cause significant changes in IgG titers (50,100, 37,700, and 34,400 after a total of 3, 6, and 9 cycles, respectively) or avidity (89, 83, and 85% after a total of 3, 6, and 9 cycles, respectively).

Following primary infection, the avidity of serum IgG was low (<30%), at least within 15 days of the onset of rash (cases 1 through 7 and 12 through 15 [Tables 1 and 2]), while the avidity was high (>75%) in SSPE patients (Table 3). Patient 8, who had been undergoing immunosuppressive therapy, showed an apparently normal humoral response. In this patient, the avidity was still <30% at day 38 from the onset of fever.

Patient 9, whose clinical presentation was indistinguishable from that of natural primary measles but who had a history of vaccination, had a slightly different pattern from patients 1 through 7 in that the IgG titer was relatively higher already at day 1 (2,600 in this patient compared with ≤300 at the same time in other patients) and was exceptionally high (52,700) at day 8. Moreover, the avidity was already 40% at day 8. Patient 10, who was suspected of having vaccine measles without Koplik spots, exhibited a regular humoral response with a positive response by IgM and IgG with low avidity. Patient 11, who had atypical measles, showed a negative response by IgM with initial high titers of IgG as expected. The avidity was consistently intermediate.

In CSF samples from patients 12 through 15 with CNS complications, neither IgM nor IgG was detected. Patient 16, who had encephalomyelitis due presumably to SVF, exhibited striking results. First, this patient had a positive IgM response. Second, the initial high avidity of serum IgG (78%) declined to 53%. Third, measles virus-specific IgG was detected in CSF, with its highest titer (20,400) being at the acute phase and with the titer profoundly decreasing to 800 thereafter. Moreover, the avidity of CSF IgG was significantly lower than that of serum IgG and declined from 23 to 5%. In case 17, similarly, initial high titers of serum IgG of high avidity were observed along with a positive response by IgM. Unlike in case 16, measles-specific IgG was not detected in CSF from this patient.

Extremely high titers of serum IgG of high avidity were observed in patients with SSPE as expected (patients 18 through 24 [Table 3]). A remarkable finding in this series was that the avidity of CSF IgG was consistently lower than that of serum IgG. While the avidity ratio (CSF/serum) varied between 40 and 75% according to the occasions of sampling, no remarkable correlations were found between the avidity ratio and the time elapsed from the onset of disease or the stage of the disease (13).

DISCUSSION

The concept of avidity was introduced into clinical practice in 1984 when a simple and reliable method of avidity testing was developed by utilizing ELISA and a mild protein-denaturing agent (12). Since then, various infectious agents have been the study subjects, and previous studies have confirmed the ability of avidity testing to clearly discriminate between primary and recurrent infection and have demonstrated an advantage over detection of IgM in that the avidity testing is free from false positives derived from cross-reactivity to other antigens or from the presence of rheumatoid factor (9, 12). Among reported methods for measuring avidity, the urea-denaturing method is simple, and we therefore utilized this method in this study of measles virus infection.

First, we sought to determine the reliability of the method. As shown in the results, low (<30%) avidity in acute-phase serum samples following primary infection and high (>75%) avidity in samples from SSPE patients were observed. These results were in complete agreement with the previous results in that the avidity was <30% following acute primary infection (2, 5, 27, 30). On the basis of these facts, we believe that the method employed in this study was reliable in discriminating

TABLE 2. Avidity of serum and CSF IgG in measles patients with CNS complications

Patient	Age	Sex[a]	Presence of Koplik spots	Type of CNS involvement	Sample	No. of days from onset of rash/CNS symptoms[b]	Result of IgM detection[c]	IgG	
								Titer (100)[d]	Avidity (%)
12	13 yr	F	+	Acute encephalitis	Serum	5/1	Pos	17	27
					CSF	7/3	Neg	ND	
13	12 yr	F	+	Acute encephalitis	Serum	6/2	Pos	13	18
					CSF	15/11	Neg	ND	
14	9 yr	M	+	Acute encephalitis	Serum	9/5	Pos	29	13
						16/12	Pos	63	9
					CSF	9/5	Neg	ND	
						16/12	Neg	ND	
15	5 yr	M	+	Acute encephalitis	Serum	7/4	Pos	52	8
					CSF	7/4	Neg	ND	
16[e]	12 yr	F	−	Acute encephalomyelitis	Serum	6/4	Pos	779	78
						32/30	Pos	734	70
						49/47	Neg	610	53
					CSF	12/10	Neg	204	23
						19/17	Neg	88	14
						35/33	Neg	8	5
17[f]	10 yr	M	−	Optic neuritis	Serum	6/−11	Pos	342	72
						15/−2	Pos	314	63
					CSF	20/3	Neg	ND	

[a] F, female; M, male.
[b] The day of onset of rash/CNS symptoms, respectively, was considered day 0.
[c] Neg, negative; Pos, positive.
[d] ND, not detected.
[e] Vaccinated at the age of 1 year 6 months. This case was described in detail elsewhere (22).
[f] Vaccinated at the age of 1 year 6 months.

between low- and high-avidity serum IgG. Inouye et al. pointed out that a kit difference might affect the results of avidity testing (12). The results above indicated that the Enzygnost kit is suitable for this purpose. Although, unfortunately, we could not determine the length of time during which the avidity remains low because we were unable to obtain serum samples after recovery from acute illness, our limited data (case 8) suggest that the avidity is still low at least 5 weeks following primary infection. This result is compatible with that obtained by Tuokko, who showed that in measles virus infection the change from low to high avidity was significant after 7 weeks (30).

Next, the five patients (patients 9 through 11, 16, and 17) with a history of vaccination (four with live attenuated and one with killed vaccine) were a matter of concern. Classically, the presence of IgM in serum has been a hallmark of primary infection or PVF (17), but recently, Erdman et al. (6) and Hidaka et al. (10) presented data suggesting that IgM can also be detected in cases of SVF and that the detection of IgM should not be equated with PVF. Our patients 16 and 17 showed a positive reaction by IgM in serum as well as initial high titers of high-avidity IgG. The latter result substantiates the suggestion that these patients had had immunological priming and therefore had SVFs. In this respect, the results of case 9 are of interest. This case is apparently a PVF on the basis of the clinical presentation. A positive IgM reaction and the initial low avidity of 16% substantiates this premise. However, the IgG titer rose to 52,700 at day 8, and moreover, the avidity was 40% at that time. This pattern of IgG response is different not only from patterns in cases 16 and 17, with initial high avidities (78 and 72%, respectively) but also from those in cases of primary infection with an initial low avidity (<30%), suggesting that patient 9 may have had a low degree of immunological priming. From these facts, we speculate that SVF comprises a wide variety of immune responses to vaccine and that one extreme mimics PVF in terms of not only clinical presentation but also serological response. Nevertheless, discrimination between PVF and SVF must have a significant implication for vaccine strategy (18, 19, 25). A survey of vaccine failure cases on the basis of avidity will provide further information.

Avidity in atypical measles was intermediate and not very high. This may represent an effect of killed vaccine.

To our knowledge, there have been no studies on the avidity of CSF IgG, presumably because it is rather unusual for agent-specific IgG to be detected in CSF, irrespective of whether it is produced intrathecally or is a leakage from blood. In fact, in five of our six cases of measles complicated by CNS involvement, there was no detectable IgG in CSF. The only exception, case 16, is therefore of great interest in some respects.

The measles-specific IgG which was detected in the CSF of patient 16 had a significantly lower avidity than serum IgG. This difference clearly indicates that the IgG in CSF did not represent a leakage of serum IgG from the blood into the CNS but was produced intrathecally. Moreover, the avidity of CSF decreased consistently thereafter, and, although more slightly, the avidity of serum IgG decreased as well. To date, a decrease in avidity within this short observation period has not been reported elsewhere. This peculiar characteristic of the IgG class antibody might have contributed to the unusual clinical presentation of measles virus infection in patient 16.

Hänninen et al. reported that in a study concerning uncomplicated measles, 9 (15%) of 59 patients had detectable levels of measles virus-specific IgG in CSF (8). On the other hand,

TABLE 3. Avidity of serum and CSF IgG in patients with SSPE

Patient	Age at onset of SSPE	Age at onset of measles	Sex[a]	Sample	Interval (stage)[b]	Result of IgM detection[c]	IgG		
							Titer (100)	Avidity (%)	Avidity ratio (%)[d]
18	7 yr	1 yr	M	Serum	5 mo	Neg	432	78	
				CSF	5 mo (I)	Neg	98	34	44
19	12 yr	3 yr	M	Serum	4 wk	Neg	330	78	
				CSF	3 wk (I)	Neg	160	56	72
20	5 yr	1 yr	M	Serum	2 mo	Neg	341	100	
				CSF	2 mo (III)	Neg	161	75	75
21	6 yr	1 yr	M	Serum	2 yr	Neg	501	89	
				CSF	2 yr (II)	Neg	78	51	57
22	8 yr	2 yr[e]	M	Serum	2 mo	Neg	1,242	86	
				CSF	8 mo (III)	Neg	190	55	
				Serum	4 yr	Neg	461	96	
				CSF	4 yr (III)	Neg	184	38	40
23	7 yr	10 mo	F	Serum	4 mo	Neg	1,094	86	
				CSF	3 mo (II)	Neg	294	42	49
				Serum	10 mo	Neg	476	86	
				CSF	7 mo (III)	Neg	102	52	
24	10 yr	1 yr	F	Serum	3 wk	Neg	863	100	
				CSF	3 wk (I)	Neg	289	58	58

[a] F, female; M, male.
[b] Interval between the onset of SSPE and sampling. Stages are as described by Jabbour et al. (13).
[c] Neg, negative; Pos, positive.
[d] Avidity of CSF IgG/avidity of serum IgG in corresponding samples.
[e] Measles vaccination (no history of natural infection).

Johnson et al. demonstrated that the detection of measles virus-specific IgG in CSF is rather exceptional even in patients with CNS manifestations (14). Some authors have proposed the concept of a subacute form of measles encephalitis occurring mainly in patients with immunosuppression (11, 16, 23, 31), with sporadic detection of measles virus-specific IgG in CSF (16). An analysis of measles virus-specific IgG in CSF is needed to further our understanding of the complex features of encephalitic episodes associated with measles, but this has been hampered by a lack of a simple and versatile methodology. In this regard, avidity testing of CSF IgG, when it is detectable, is highly promising because of its simplicity and reliability.

In patients with SSPE, the avidity of CSF IgG was consistently lower than that of serum IgG (IgG avidity ratio, CSF/serum, 40 to 75%). Since this ratio did not change over time, it is reasonable to consider that within the CNS of SSPE patients, maturation of the intrathecally produced IgG does not occur as it does in serum. Although it was lower than that of serum IgG, unlike in case 16, the level of avidity, along with the high titer, of CSF IgG was maintained for years after the onset of CNS symptoms in SSPE patients. This may reflect a difference between the transient antigenic stimulus in the CNS of patient 16 and the continuous antigenic stimuli in the CNSs of SSPE patients. Overall, if the consistently low avidity of intrathecally produced IgG compared with that of serum IgG is a common finding with other infectious agents, irrespective of whether the CNS infection is acute or chronic, the avidity testing of CSF IgG may be used as a more direct indicator than an IgG index (29) of intrathecal production of IgG.

In conclusion, the avidity testing using a commercially available ELISA kit with a simple urea treatment method is reliable and highly informative in the analysis and understanding of measles virus infection.

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